In some cases, the immunoassay method may be employed directly to detect any of the elements of the group antigen, antibody or complement. In the direct method an incomplete mixture lacking but one element of the group antigen, antibody or complement is prepared and the presence of the missing element in a test sample is assessed by the extent to which addition of the sample to the incomplete mixture promotes lysis of the liposome by immune specific attack on the liposomal membrane or exposure of the encapsulated enzyme to the fluid around the liposome. When the test material is to be tested for the cognate antibody or antigen to that which acts as a label for the liposome, no antigen or antibody need be added to the mixture. A direct immunoassay method for antigen or antibody would comprise a mixture of:

(a) liposomes labeled with one of an antigen or its cognate antibody, carrying an enzyme and having a signal to noise of no less than 10,
(b) a substrate for said enzyme,
(c) a test material to be tested for the one antigen or cognate antibody,
(d) complement.

If the aim of a direct immunoassay is to assess the active complement in a test sample the method would comprise a mixture of:

(a) liposomes labeled with one of an antigen or its cognate antibody, carrying an enzyme and having a signal to noise of no less than 10,
(b) a substrate for said enzyme,
(c) a test material to be tested for complement,
(d) free cognate of the other of said one antigen or antibody.

A preferred immunoassay method preferably comprises, forming a mixture of:

(a) liposomes labeled with one of an antigen or its cognate antibody carrying an enzyme and having a signal to noise ratio of no less than 10,
(b) a substrate for said enzyme,
(c) a test material to be tested for the one antigen or cognate antibody,
(d) complement, and
(e) free cognate of the other of said one antigen or antibody,

and detecting the presence or absence of enzymatic activity in said mixture. In this method the antigen to be tested for can be used to label the liposomes and free cognate antibody used. Alternately the antibody to be tested for can be used to label the liposome and free cognate antibody used.

Preferably the method is carried out as a one-step method and all materials are added to a single vial with incubation at standard immunological conditions as for example 37° C., or in a range of from 4° C. to 45° C. for periods of from about 1 second to 120 minutes. Alternately all but the enzyme substrate are admixed and incubated for 5 to about 120 minutes or more and than this admixture is added to an enzyme substrate and the result determined.

A kit for detecting one of an antigen or its cognate antibody preferably has a first container carrying liposomes labeled with one of the antigen or its cognate antibody suspended in an appropriate buffer. A second container carries powdered lyophilized or frozen concentrated antibody or antigen which is the cognate of that on the liposome. A third vial carries powdered or frozen concentrated complement which can be in the form of guinea pig serum and a fourth container carries an enzyme substrate for the enzyme which may be in liquid or powder form. Buffer is also included in another container.

A one-step method can be used where all components are mixed and incubated. However, in some cases, the procedure may be carried out in two or more steps with some of the materials incubated together prior to complete mixing. In all cases, no separation is carried out after the immune reaction or absence of it and a direct reading is made of the reaction materials to determine the presence or absence of the antigen or antibody in the test specimen, by detecting enzyme activity or reaction with the substrate.

It is a feature of this invention that the test can be carried out quickly by untrained personnel at relatively low cost. The readout can be subjective, e.g., visual as by a color change when qualitative readouts are desired. Semi-quantitative readouts may be obtained subjectively, as when deep to light color changes may occur. Spectrophotometric methods and the like can also be used to detect the presence or absence of enzymatic activity in the presence of substrate which indicates lysing of the liposome or immune specific attack on the liposomal membrane so as to expose the enzyme to the substrate. The exposure of enzyme activity will occur when the immune reaction occurs to form an immune complex and affect the bilayer or enzyme enclosing membrane of the liposomes. When the antibody or antigen, as the case may be, in the system reacts with the opposite with which the liposome is labeled in the presence of active complement, the enzyme is released. However, if the test sample contains the antigen or antibody to be tested for, reaction of the cognate in the media prevents or reduces reaction with the cognate label and thus prevents the enzyme from being detected in the substrate indicating a positive for the antigen or antibody being tested for.

BRIEF DESCRIPTION OF PREFERRED EMBODIMENTS

The liposomes of the present invention are sometimes called smectic mesophases or synthetic vesicles. They are in fact dry lipid films suspended in aqueous media as have been described by Uemura, K. and Kinsky, S. C. (1972) Biochemistry 11, 4085-4094. Liposomes are believed to consist of lipid bilayers which separate an internal aqueous compartment from an external aqueous media and are in fact prototypes of biological membranes. The liposomes mimic the properties of biological membranes. As is known, they can be made to contain either enzyme substrates or enzymes. For purposes of the present invention, the liposomes contain an enzyme and have an outer surface substantially free of the enzyme which outer surface encloses the enzyme such that the catalytic action of the enzyme is not detectable unless the outer surface encapsulating the membrane is disrupted and is labeled with an antigen or its cognate antibody depending upon the test to be carried out. Preferably if one is testing for the antibody, the liposome will be labeled with that antibody while if one is testing for the antigen, the liposome will be labeled with the antigen.

Liposomes have been known in the art. However, the art is not believed to have previously obtained liposomes having enzymes contained therein which liposomes have signal to noise ratios of no less than 5. This is probably so since the art has not recognized the ad-